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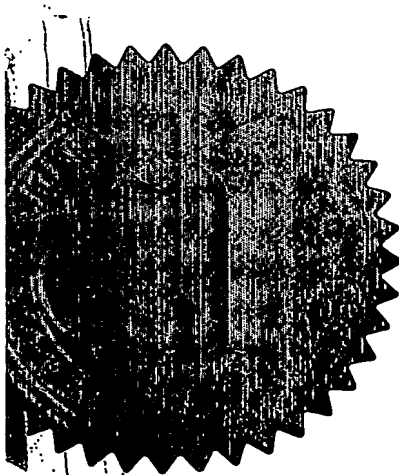
Application No. 2003/0761

Date of Filing 14 October 2003

Applicant THE PROVOST, FELLOWS AND SCHOLARS
OF THE COLLEGE OF THE HOLY AND
UNDIVIDED TRINITY OF QUEEN ELIZABETH,
NEAR DUBLIN, a registered charity of College
Green, Dublin 2, Ireland.

Dated this 19 day of October 2004.

An officer authorised by the
Controller of Patents, Designs and Trademarks.



REQUEST FOR THE GRANT OF A PATENT

PATENTS ACT, 1992

The Applicant(s) named herein hereby request(s)

 X the grant of a patent under Part II of the Act
 the grant of a short-term patent under Part III of the Act on the basis of the information furnished hereunder.

1. Applicant(s)

Name THE PROVOST, FELLOWS AND SCHOLARS
OF THE COLLEGE OF THE HOLY AND UNDIVIDED TRINITY
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Description/Nationality

A registered charity

2. Title of Invention

"A method"

3. Declaration of Priority on basis of previously filed application(s) for same invention (Sections 25 & 26)

Previous filing date

Country in or for
which filed

Filing No.

4. Identification of Inventor(s)

Name(s) of person(s) believed
by Applicants(s) to be the inventor(s)

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5. Statement of right to be granted a patent (Section 17(2) (b))

The Applicant derives the rights to the Invention by virtue of a Deed of Assignment dated October 13, 2003.

6. Items accompanying this Request – tick as appropriate

- (i) X Prescribed filing fee (€125.00)
- (ii) X Specification containing a description and claims
 Specification containing a description only
 X Drawings referred to in description or claims
- (iii) An abstract
- (iv) Copy of previous application(s) whose priority is claimed
- (v) Translation of previous application(s) whose priority is claimed
- (vi) X Authorisation of Agent (this may be given at 8 below if this Request is signed by the Applicant (s))

7. Divisional Application (s)

The following information is applicable to the present application which is made under Section 24 –

Earlier Application No:

Filing Date:

8. Agent

The following is authorised to act as agent in all proceedings connected with the obtaining of a patent to which this request relates and in relation to any patent granted -

Name

John A. O'Brien & Associates

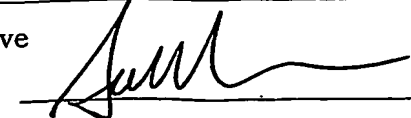
Address

The address recorded for the time being in the Register of Patent Agents, and currently Third Floor, Duncairn House, 14 Carysfort Avenue, Blackrock, Co. Dublin, Ireland.

9. Address for Service (if different from that at 8)

As above

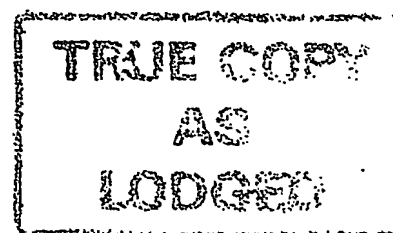
Signed



JOHN A. O'BRIEN & ASSOCIATES

Date October 14, 2003

A method

Introduction

5 The invention relates to filamentous haemagglutinin (FHA) or a derivative or mutant or fragment or variant or peptide thereof.

10 Cells of the innate immune system, especially dendritic cells (DC), direct the differentiation of naïve CD4⁺ T cells into functionally distinct Th1, Th2 or regulatory T (Tr) cell subtypes. Activation of immature DC through binding of conserved microbial molecules to pathogen recognition receptors (PRRs), such as Toll-like receptors (TLR) and integrins, is accompanied by maturation and homing to the lymph nodes, where the mature DC presents antigen to the naïve T cells. Activation of DC by pathogen derived molecules plays a critical role in regulating the
15 differentiation of naïve CD4⁺ T cells into distinct T cell subtypes (1, 2). Th1 cells confer protection against intracellular infection but are also associated with inflammatory responses and autoimmune disease, whereas Th2 cells are involved in allergic responses. Tr cells are capable of suppressing Th1 and Th2 responses.

20 *Bordetella pertussis* causes a protracted and severe disease, which is often complicated by secondary infection and pneumonia, and can have a lethal outcome in young children. Recovery from infection is associated with the development of *B. pertussis*-specific Th1 cells and these cells play a critical role in clearance of the bacteria from the respiratory tract. However, antigen-specific Th1 responses in the
25 lung and local lymph nodes, are severely suppressed during the acute phase of infection. *B. pertussis* has evolved a number of strategies to circumvent protective immune responses.

30 The virulence factor, filamentous haemagglutinin (FHA) from *B. pertussis*, is capable of inhibiting LPS-driven IL-12 production by macrophages, IL-12 and IFN- γ production in a murine model of septic shock (3) and Th1 responses to an unrelated

pathogen, influenza virus, when administered simultaneously to the respiratory tract (4). FHA is considered to function primarily as an adhesin, mediating binding of *B. pertussis* to the $\beta 2$ -integrin (CR3, CD11b/CD18, $\alpha M\beta 2$) via binding to leukocyte response integrin ($\alpha V\beta 3$, CD61) and the integrin-associated protein (CD47) complex (5). FHA may also contribute to suppressed Th1 responses during acute infection with *B. pertussis* by the induction of T cells with regulatory activity, as a result of its interaction with cells of the innate immune system. FHA interacts directly with DC to induce IL-10 and inhibit LPS-induced IL-12 and inflammatory chemokine production (6). The DC generated following interaction with FHA selectively stimulates the induction of Tr1 cells from naïve T cells. Tr1 clones specific for FHA and pertactin (PRN) from *B. pertussis* were generated from the lungs of acutely infected mice. These Tr1 cells secreted high levels of IL-10 and inhibited protective Th1 responses against *B. pertussis* *in vitro* and *in vivo* (6). These findings demonstrated a novel function for Tr1 cells, exploited by a respiratory pathogen to evade protective immunity, and provided evidence that these regulatory cells are induced by DC in which IL-10 production is activated and IL-12 suppressed following interaction with a pathogen-derived molecule.

Multiple sclerosis (MS) is an autoimmune disease that affects the central nervous system. Individuals with this disease have autoreactive T cells (T cells that recognize self antigens), which together with interleukin (IL)- 1β and tumour necrosis factor (TNF) α , participate in the formation of inflammatory lesions along the myelin sheath of nerve fibres. The cerebrospinal fluid (CSF) of patients with MS contains activated T cells, which infiltrate the brain tissue and cause the characteristic inflammatory lesions, destroying the myelin. Experimental autoimmune encephalomyelitis (EAE) is an animal model for MS. It is induced in mice or rats by injection of myelin basic protein (MBP) or myelin oligodendrocyte glycoprotein (MOG) or peptides thereof with complete Freund's adjuvant. The disease can also be induced by transfer of MBP or MOG-specific T cells that secrete IFN- γ (called Th1 cells). The animals develop cellular infiltration of the myelin sheaths of the central

nervous system, resulting in demyelination and eventually paralysis. The clinical signs and pathological changes resemble MS.

5 Crohn's disease and ulcerative colitis are inflammatory bowel diseases in humans. These autoimmune diseases are inflammatory conditions of the intestine mediated by CD4⁺ T cells. Regulatory T cells (Tr cells) prevent the development of autoimmune diseases in normal individuals. Injection of CD45RB^{high} (naïve) T cells can induce colitis in severe combined immunodeficient (SCID) mice, which can be prevented by co-transfer of CD45RB^{low} or CD4⁺ CD25⁺ regulatory T cells (7). Furthermore
10 elimination of CD45RB^{low} or CD4⁺ CD25⁺ regulatory T cells leads to spontaneous development of various autoimmune diseases in otherwise normal mice or rats (8).

A method of modulating the induction of Tr cells *in vivo* would have valuable potential for the treatment of inflammatory and autoimmune diseases and allergy.
15

Statements of Invention

According to the invention there is provided a method for the prophylaxis and/or
20 treatment of an immune-mediated disorder comprising the step of administering an agent comprising filamentous haemagglutinin (FHA) or a derivative or mutant or fragment or variant or peptide thereof.

Preferably the filamentous haemagglutinin (FHA) is derived from *Bordetella*
25 *pertussis* or *Bordetella bronchiseptica* or *Bordetella parapertussis*.

In one embodiment of the invention the agent promotes the generation of Tr cells in response to a self antigen.

30 In another embodiment of the invention FHA acts as an adjuvant *in vivo* to promote the induction of Tr cells to co-administered self or foreign antigens.

Preferably the self antigen is a myelin basic protein. Most preferably the myelin basic protein is myelin oligodendrocyte glycoprotein (MOG) synthetic peptide.

5 In one embodiment of the invention the immune-mediated disorder is multiple sclerosis.

10 In another embodiment of the invention the immune-mediated disorder is selected from any one or more of multiple sclerosis, Crohn's disease, inflammatory bowel disease, type 1 diabetes or rheumatoid arthritis.

In one embodiment of the invention the immune-mediated is asthma or atopic disease.

15 In one aspect of the invention the agent is in a form for oral, intranasal, intravenous, intradermal, subcutaneous or intramuscular administration.

20 The invention also provides a pharmaceutical composition comprising FHA or derivative or mutant or fragment or variant or peptide thereof.

The invention further provides a pharmaceutical composition comprising FHA or derivative or mutant or fragment or variant or peptide thereof as adjuvant for immunization with a self or foreign antigen.

25 The invention also provides a vaccine for the treatment of immune-mediated disorders comprising FHA or a derivative or mutant or fragment or variant or peptide thereof.

30 The invention further provides antibodies to FHA or a derivative or mutant or fragment or variant or peptide thereof.

One aspect of the invention also provides use of an agent comprising FHA or a derivative or mutant or fragment or variant or peptide thereof or product of cells activated by the agent for the prophylaxis of an immune-mediated disorder.

5 Another aspect of the invention provides use of an agent comprising FHA or a derivative or mutant or fragment or variant or peptide thereof or product of cells activated by the agent for the prophylaxis and/or treatment of multiple sclerosis.

10 The invention also provides use of an agent comprising FHA or a derivative or mutant or fragment or variant or peptide thereof or product of cells activated by the agent for the prophylaxis and/or treatment of a disease selected from any one or more of multiple sclerosis, Crohn's disease, inflammatory bowel disease, type 1 diabetes or rheumatoid arthritis. Preferably the disease is Crohn's disease or inflammatory bowel disease.

15 The invention further provides use of an agent comprising FHA or a derivative or mutant or fragment or variant or peptide or product of cells activated by the agent for the prophylaxis and/or treatment of asthma or allergy.

20 The term derivative or mutant or fragment or variant or peptide as used herein are understood to include any molecule or macromolecule consisting of a functional portion of FHA.

25 The term antigen is taken throughout to mean any substance that binds specifically to an antibody or T cell receptor. The term self- or auto-antigen is taken to mean an endogenous antigen on self-tissue in the body, which is not foreign. The term foreign antigen is taken to mean an antigen from a pathogen (bacteria, virus or parasite).

30

Brief Description of the Invention

The invention will be more clearly understood from the following description thereof, given by way of example with reference to the accompanying drawings in which: -

Fig. 1 is a graph showing the effect of immunization with myelin oligodendrocyte (MOG) peptides with FHA on the disease progression (average disease index) in experimental autoimmune encephalomyelitis (EAE), a murine model for multiple sclerosis. Mice were immunized subcutaneously (s.c.) with 50 μ g MOG peptide (residues 35-55) and 5.0 μ g FHA in phosphate buffered saline. This was repeated 21 days later. Control mice received MOG peptide or saline only. 7 days after the second immunization, EAE was induced by s.c. administration of 150 μ g MOG peptide emulsified in complete Freund's adjuvant, supplemented with 1 mg *Mycobacteria tuberculosis* intraperitoneal (i.p.) injection of 500 ng pertussis toxin, followed 2 days later by a second i.p. injection with 500 ng pertussis toxin. Mice were assessed daily for clinical signs of EAE, and scored as follows: 1 = tail paralysis, 2 = wobbly gait, 3 = hind limb weakness, 4 = hind limb paralysis, 5 = complete paralysis of hind and fore limbs, 6 = death. The disease index was calculated by adding all daily average disease scores, dividing the average day of onset, and multiplying by 100;

Fig. 2 is a graph showing the effect of immunization with FHA and MOG peptide on average disease score over time in experimental autoimmune encephalomyelitis (EAE), in a murine model for multiple sclerosis;

Fig. 3 is a graph showing histopathology section of spinal cords of mice after induction of EAE (untreated) or after immunization with myelin

oligodendrocyte peptide (MOG) or MOG peptide + FHA (MOG + FHA). EAE was induced and mice immunized as described in Fig. 1, sections of spinal cord were removed from mice 19-23 days after induction of EAE and stained with haematoxylin and eosin. The EAE induced in un-treated and MOG-immunized mice is severe with a pronounced mononuclear cell infiltrate; immunized with MOG and FHA prevents mononuclear cell infiltrate, encephalitis, perivascular cuffing and demyelination; and

Fig. 4 is a graph showing the effect of s.c. administration of FHA on the development of intestinal inflammation in a murine colitis model. Groups of 6 severe combined immunodeficient mice (SCID) mice were injected intravenously with CD45RB^{hi} naïve T cells alone or with CD45RB^{low} T cells or were injected with CD45RB^{hi} naïve T cells with FHA administered s.c. (10 µg / mouse 2 weeks apart). Body weight was recorded and mice were sacrificed after 8-12 weeks. Colon weights were recorded and histology was performed on hematoxylin and eosin stained sections of the colons. Administration of CD45RB^{hi} cells was associated with the development of severe intestinal inflammation in SCID mice, which was accompanied by severe weight loss. Transfer of CD45RB^{low} cells prevented inflammation and weight loss. Furthermore s.c. therapy with FHA prevented colon inflammation and weight loss; FHA treated mice had a marked reduction of intestinal inflammation, reduced colon weights and less colon shrinkage than control CD45RB^{hi} transferred mice given no treatment.

Detailed description

We have found that filamentous haemagglutinin (FHA) from *Bordetella pertussis* can be used as an adjuvant in a vaccine against autoimmune disease. Parenteral immunisation of mice with the myelin oligodendrocyte glycoprotein (MOG)

synthetic peptide in the presence of FHA was found to prevent the developments of disease symptoms and pathology in experimental allergic encephalomyelitis (EAE), a murine model for multiple sclerosis. Immunisation with self or foreign antigens in the presence of FHA promotes the induction of regulatory T cells specific for the bystander antigen and these T cells appear to be capable of preventing self- reactive immune responses leading to autoimmune conditions.

Current approaches for the treatment of multiple sclerosis have focused on therapeutic strategies aimed at reducing inflammation in the brain of individuals who have already started to develop disease symptoms.

We have found that FHA may be used to prevent the onset of clinical signs of EAE by inducing memory T cells with suppressor activity and are specific for myelin proteins.

We also found that s.c. administration of FHA reduced the intestinal inflammation, reduced colon weight gain and shrinkage and prevented weight loss induced in SCID mice by transfer of naïve CD45RB^{hi} cells. These data suggests that FHA can prevent the development of autoimmune diseases, possibly by the induction of regulatory T cells or by the production of innate IL-10, which promotes the induction of regulatory T cells or has a direct suppressive effects on the immune responses that mediate autoimmune diseases.

FHA has already been approved for use in humans and is currently a component of several acellular pertussis vaccines, where it is absorbed to aluminium hydroxide.

FHA or derivatives thereof may be used in the treatment of, or as a component of a vaccine in the prevention of immune mediated diseases, including but not limited to multiple sclerosis, Crohn's disease, inflammatory bowel disease, type 1 diabetes and rheumatoid arthritis.

FHA or derivatives thereof may also be used in treatment of, or as a component of a vaccine in the prevention of asthma or atopic diseases.

Many of the diseases detailed above have no satisfactory treatment and in most cases steroids and non-steroidal anti-inflammatory drugs are employed. However, these are non-specific and have side effects. More recently drugs that inhibit key inflammatory cytokines, in particular tumour necrosis factor (TNF)- α , have been developed. These include antibodies or soluble TNF receptors that are effective against certain autoimmune diseases, but are associated with side effects (including recurrent tuberculosis) and are limited to diseases where TNF- α is the key mediator of pathology. Another therapeutic approach is the direct administration of anti-inflammatory cytokines (e.g. IL-10), but this is compromised by the short half-life of the cytokines *in vivo*. Alternative strategies could employ agents that induce anti-inflammatory cytokines, such as IL-10, which will have a direct immunosuppressive effect *in vivo*.

Molecules that promote the induction of suppressor or regulatory T cells, have the potential to limit inflammatory and Th1-mediated immune responses. FHA has the potential to drive innate and adaptive IL-10 and thereby act as an immunotherapeutic drug or as an adjuvant for vaccines to prevent immune mediated disease.

The invention will be more clearly understood by the following examples.

Examples

Bordetella pertussis was grown for 3 days on Bordet-Gengou agar plates. The colonies, which were hemolytic, were used to start a liquid preculture (30 ml) in Stainer-Scholte (SS) medium, supplemented with dimethyl-beta cyclodextrin (CDX; purchased from Sigma) at a final concentration of 0.5 g per liter (CDX induces the release of FHA from the bacterial surface). This pre-culture was grown overnight at 37 °C under agitation and used to inoculate a large cultures (250 ml of SS medium in

1-L flasks). This culture was grown at 37°C under agitation for 36-48 hours. Once the plateau phase was reached (determined by measuring optical density of the culture), the cells in culture medium was centrifuged at 7000 rpm for 20 min at 4°C and the supernatant collect. The FHA was purified from the supernatant using FPLC with a matrix of heparin-sepharose column (Amersham) equilibrated with PBS pH 7.4. After loading the sample, the column was washed with PBS and eluted with PBS supplemented with 0.5 M NaCl at room temperature using a flow rate of 2 ml/minute. The fractions with the peak elution contained the FHA. Contaminating LPS and was removed on endotoxin-removal columns (Detoxi-Gel™ endotoxin removing gel; Pierce, Rockford, IL, USA). Following this step, endotoxin was undetectable in the preparation using the chromogenic limulus amebocyte lysate (LAL) assay (Bio Whittaker, Walkersville, MD, USA).

Murine model for multiple sclerosis

Experimental autoimmune encephalomyelitis (EAE) is a murine model for multiple sclerosis. EAE is induced in C57BL/6 mice by s.c. administration of 150 µg MOG peptide emulsified in complete Freund's adjuvant, supplemented with 1 mg *Mycobacteria tuberculosis* intraperitoneal (i.p.) injection of 500 ng pertussis toxin, followed 2 days later by a second i.p. injection with 500 ng pertussis toxin. Mice develop symptoms of paralysis. In experiments to assess the effects of FHA as a adjuvant for a vaccine against autoimmune disease, mice were immunized subcutaneously (s.c.) with 50 µg MOG peptide (residues 35-55) and 5.0 µg FHA in phosphate buffered saline. This was repeated 21 days later. Control mice received MOG peptide or saline only. 7 days after the second immunization. Mice were assessed daily for clinical signs of EAE, and scored as follows: 1 = tail paralysis, 2 = wobbly gait, 3 = hind limb weakness, 4 = hind limb paralysis, 5 = complete paralysis of hind and fore limbs, 6 = death.

Table 1 shows the disease score and disease index results. The results indicate that the administration of FHA as an adjuvant significantly inhibits disease progression.

Immunization Group	Incidence	Day of onset	Mean Max Clinical Score	Disease Index at day 23
Control	10/11	16.4	2.9	195
MOG	7/8	15	1.875	100
MOG + FHA	5/8	20.5	0.625	5

Table 1

Incidence is the number of mice out of the number tested that develop any clinical symptoms of EAE. The disease index was calculated by adding all daily average disease scores, dividing the average day of onset, and multiplying by 100.

Murine model for colitis in humans

In a murine model for colitis in humans, CD45RB^{hi} naïve T cells were injected into severe combined immunodeficient (SCID) mice. This results in the development of chronic colonic inflammation 6-8 weeks after injection. Histology was characterized by influx of mononuclear cells in all layers of the intestinal wall, hyperplasia and decreased differentiation of intestinal epithelial cells. Groups of 6 SCID mice were injected intravenously with CD45RB^{hi} naïve T cells alone or with CD45RB^{low} T cells or were injected with CD45RB^{hi} naïve T cells with FHA administered s.c. (10 µg / mouse 2 weeks apart). Body weight was recorded and mice were sacrificed after 8-12 weeks. Colon weights were recorded and histology was performed on hematoxylin and eosin stained sections of the colons.

The invention is not limited to the embodiments hereinbefore described which may be varied in detail.

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2. Lavelle, E., E. McNeela, M. E. Armstrong, O. Leavy, S. C. Higgins, and K. H. G. Mills. 2003. Cholera toxin promotes the induction of regulatory T cells as well as Th2 cells specific for bystander antigens by modulating dendritic cell activation. *J. Immunol.* 171:2384-2392.
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5. McGuirk, P., P.A. Johnson, E.J. Ryan, and K.H.G. Mills. 2000. Filamentous hemagglutinin and pertussis toxin from *Bordetella pertussis* modulate immune responses to unrelated antigens. *J. Infect. Dis.* 182, 1286-1289.
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Claims

1. A method for the prophylaxis and/or treatment of an immune-mediated disorder comprising the step of administering an agent comprising filamentous haemagglutinin (FHA) or a derivative or mutant or fragment or variant or peptide thereof.
5
2. A method as claimed in claim 1 wherein the filamentous haemagglutinin (FHA) is derived from *Bordetella pertussis* or *Bordetella bronchiseptica* or *Bordetella parapertussis*.
10
3. A method as claimed in claim 1 or 2 wherein the agent promotes the generation of Tr cells in response to a self antigen.
4. A method as claimed in any preceding claim wherein FHA acts as an adjuvant *in vivo* to promote the induction of Tr cells to co-administered self or foreign antigens.
15
5. A method as claimed in claim 3 or 4 wherein the self antigen is a myelin basic protein.
20
6. A method as claimed in claim 5 wherein the myelin basic protein is myelin oligodendrocyte glycoprotein (MOG) synthetic peptide.
7. A method as claimed in any preceding claim wherein the immune-mediated disorder is multiple sclerosis.
25
8. A method as claimed in any preceding claim wherein the immune-mediated disorder is selected from any one or more of multiple sclerosis, Crohn's disease, inflammatory bowel disease, type 1 diabetes or rheumatoid arthritis.
30

9. A method as claimed in any preceding claim wherein the immune-mediated is asthma or atopic disease.
- 5 10. A method as claimed in any preceding claim in wherein the agent is in a form for oral, intranasal, intravenous, intradermal, subcutaneous or intramuscular administration.
- 10 11. A pharmaceutical composition comprising FHA or derivative or mutant or fragment or variant or peptide thereof.
12. A pharmaceutical composition comprising FHA or derivative or mutant or fragment or variant or peptide thereof as adjuvant for immunization with a self or foreign antigen.
- 15 13. A vaccine for the treatment of immune-mediated disorders comprising FHA or a derivative or mutant or fragment or variant or peptide thereof.
- 20 14. Antibodies to FHA or a derivative or mutant or fragment or variant or peptide thereof.
15. Use of an agent comprising FHA or a derivative or mutant or fragment or variant or peptide thereof or product of cells activated by the agent for the prophylaxis of an immune-mediated disorder.
- 25 16. Use of an agent comprising FHA or a derivative or mutant or fragment or variant or peptide thereof or product of cells activated by the agent for the prophylaxis and/or treatment of multiple sclerosis.
- 30 17. Use of an agent comprising FHA or a derivative or mutant or fragment or variant or peptide thereof or product of cells activated by the agent for the

prophylaxis and/or treatment of a disease selected from any one or more of multiple sclerosis, Crohn's disease, inflammatory bowel disease, type 1 diabetes or rheumatoid arthritis.

- 5 18. Use of an agent comprising FHA or a derivative or mutant or fragment or variant or peptide thereof or product of cells activated by the agent for the prophylaxis and/or treatment of Crohn's disease.
- 10 19. Use of an agent comprising FHA or a derivative or mutant or fragment or variant or peptide thereof or product of cells activated by the agent for the prophylaxis and/or treatment of inflammatory bowel disease.
- 15 20. Use of an agent comprising FHA or a derivative or mutant or fragment or variant or peptide or product of cells activated by the agent for the prophylaxis and/or treatment of asthma or allergy.

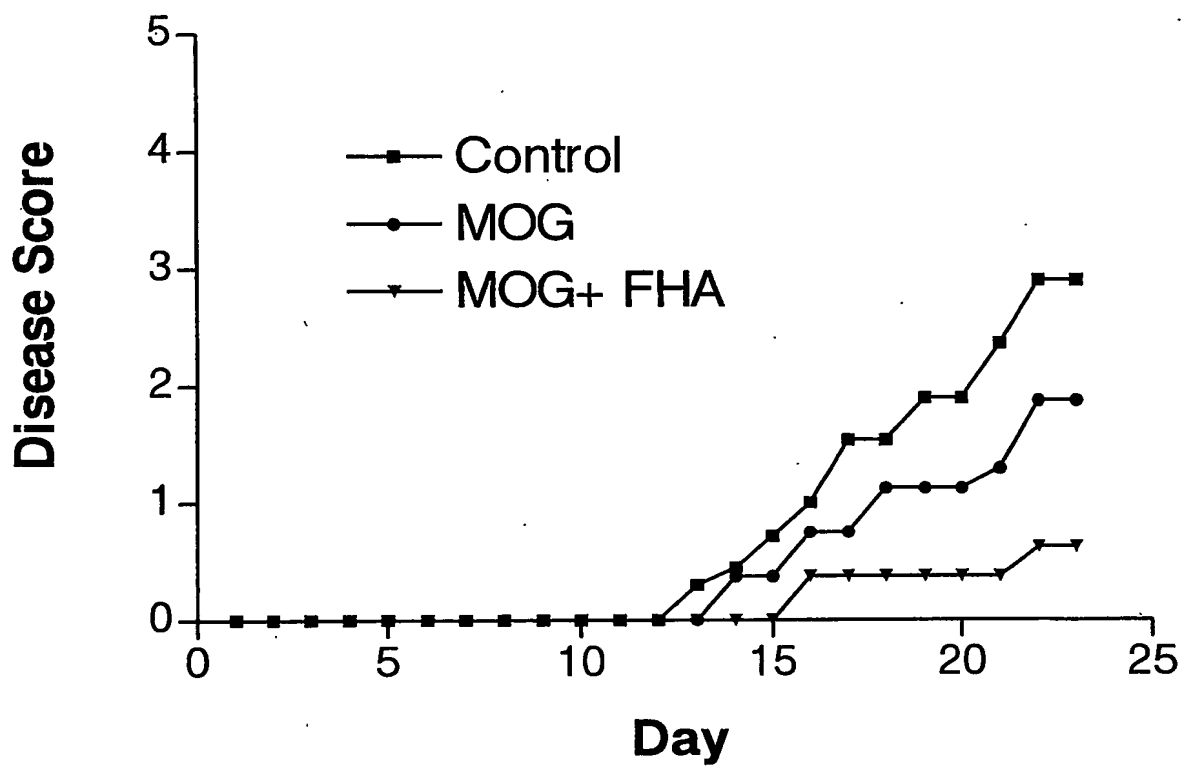


Fig. 1

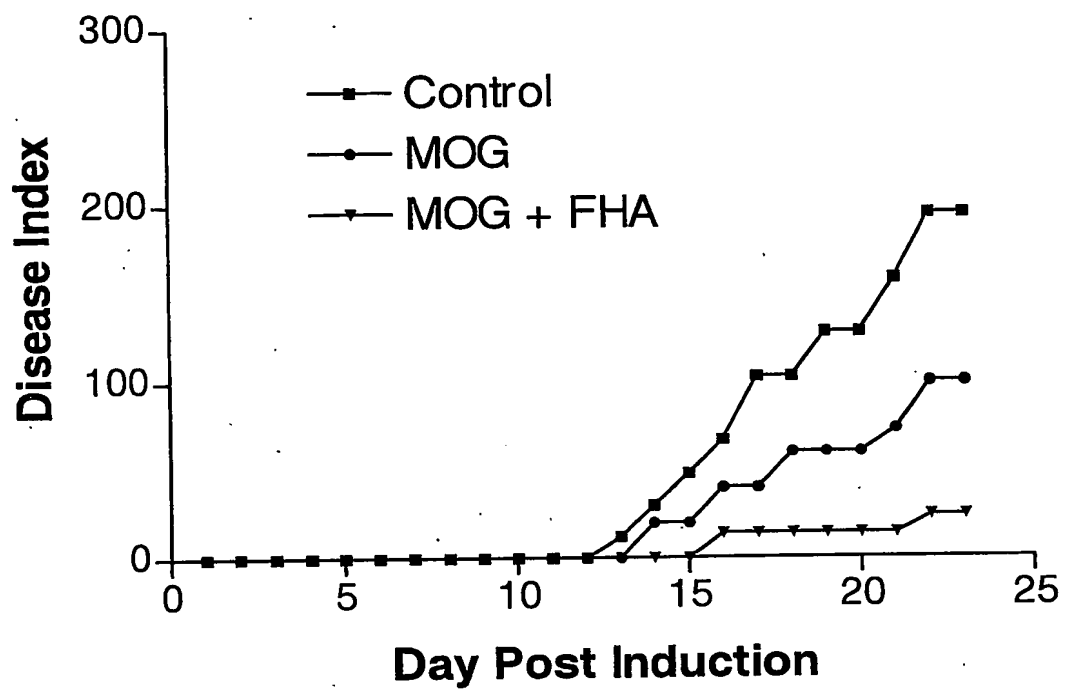


Fig. 2

Immunization with MOG and FHA prevents brain encephalitis associated with the induction of EAE

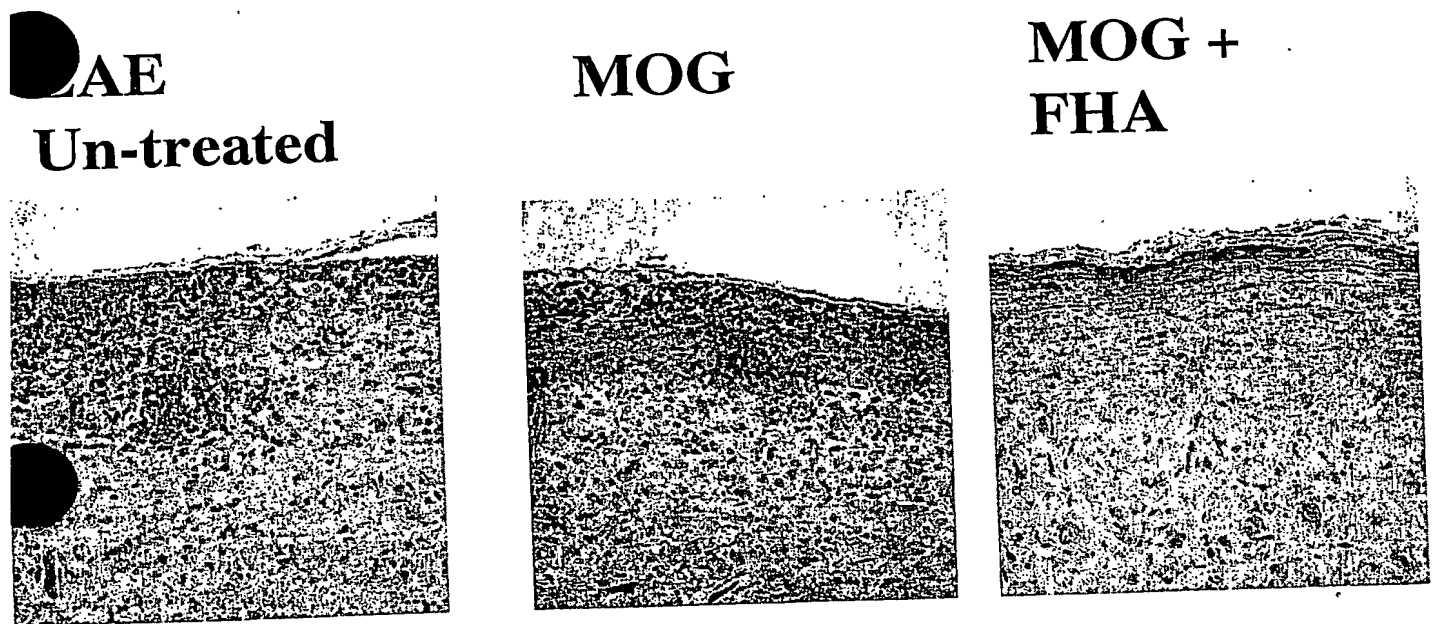
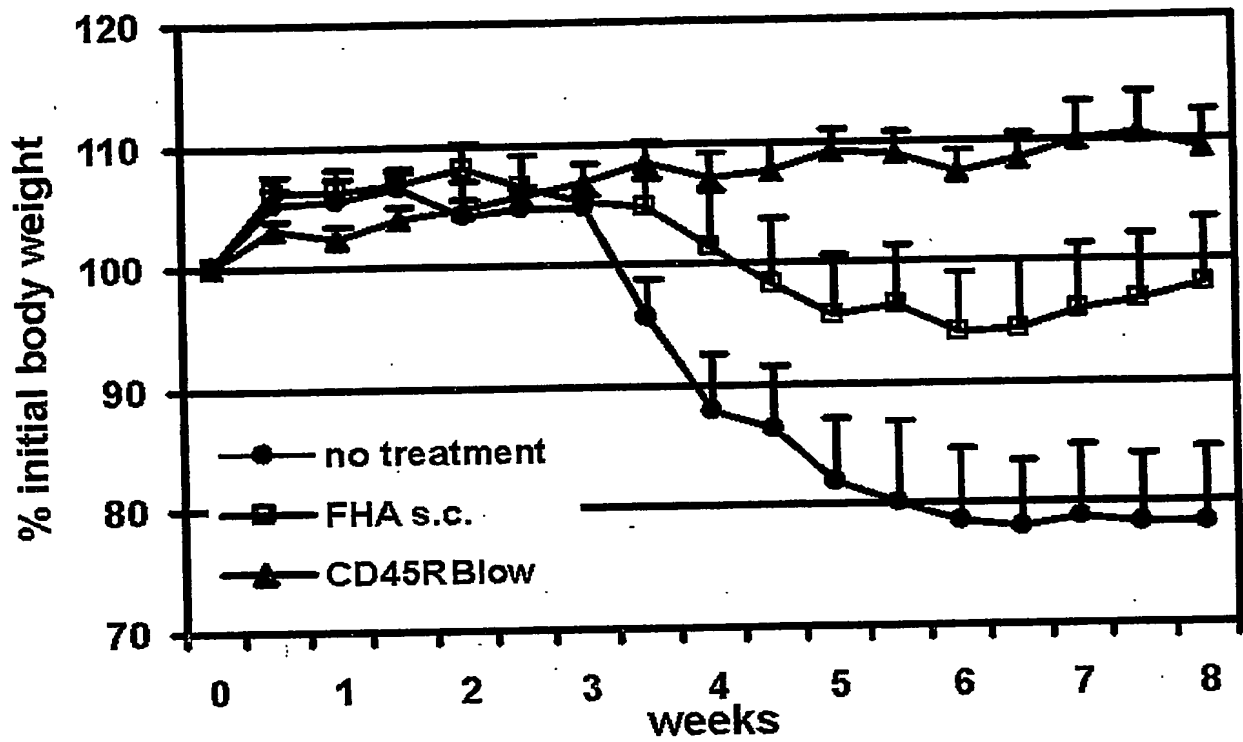


Fig. 3

A



B

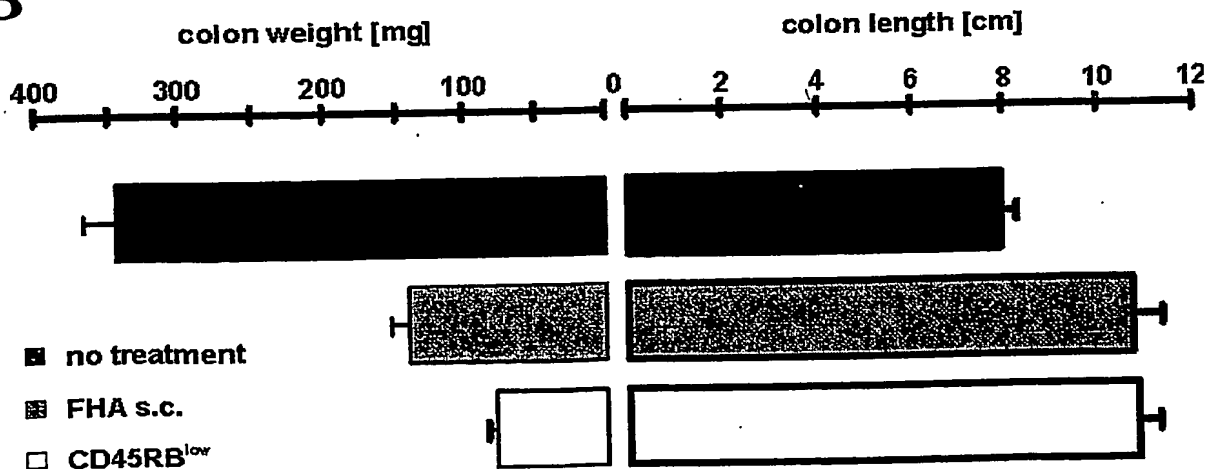


Fig. 4

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